CLAIMS

1) Circular recombinant plasmid DNA construct encoding a) a protein tag, b) a visual marker protein, and containing c) a multiple cloning site suitable for insertion of an additional gene, characterised in that the gene sequence encoding the protein tag and the visual marker protein are specifically designed and engineered at the DNA level for respectively a) immobilisation purposes and b) visualisation and quantification purposes at the protein level.

2) Construct according to claim 1, characterised in that the protein tag is chosen from the group containing lysine (lys), histidine (his), tyrosine (tyr), phenylalanine (phe), arginine (arg), glutamic acid (glu), aspartic acid (asp), glutamate, aspartate, asparagine (asn), glycine (gly), glutamine (gln), alanine (ala), valine (val), tryptophan (trp).

3) Construct according to claim 1 or 2, characterised in that the protein tag is a histidine-tag such as a polyhistidine variant, in particular (6X) histidine.

4) Construct according to any one of the preceding claims, characterised in that the visual marker protein is chosen from the group containing fluorescent or phosphorescent proteins.

5) Construct according to claim 4, characterised in that the fluorescent protein is chosen from the group containing Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), Yellow Fluorescent Protein (YFP) and Blue Fluorescent Protein (BFP) as well as their variants and/or mutants.

6) Construct according to any one of the preceding claims, characterised in that the multiple cloning site contains restriction enzyme recognition sites.

7) Construct according to claim 6, characterised in that the restriction enzyme recognition site is chosen from the group containing SacI, Sal I, Hid III, Eag I, Not I.

8) Construct according to any one of the preceding claims, characterised in that it further contains a frame adapter of variable length between the visual marker and protein tag genes.

9) Protein expressed by circular recombinant plasmid DNA construct according to any one of claims 1 to 8, characterised in that in the MCS adjacent to the visual marker, it further contains an additional target protein and in that the tag is suitable to interact directly with appropriate surface pendant groups of a support material.

10) Protein according to claim 9 characterised in that it is a fusion protein.

11) Use of the protein constructs of claim 9 or 10, in immobilisation and visualisation of proteins on compatible support material.

12) Method for preparing and immobilising a protein on a support material, characterised in that it contains the steps of:

a) Engineering at the DNA level, in series a protein tag suitable to interact directly with appropriate surface pendant groups of a support material, a fluorescent marker protein for visualisation and quantification purposes at the protein level and a multiple cloning site suitable for insertion of a target protein to be immobilised,

b) Inserting the corresponding gene of the target protein to be immobilised into the multiple cloning site;

c) Initiating protein expression.

d) Optionally pre-treating the support material;

e) Incubating the protein and support material together, wherein the protein is immobilised to the support via specific tag-surface interactions;

f) Washing away the non-specific biomolecules;

g) Optionally quantifying the fluorescence of the visual marker protein;

h) Optionally desorbing the target protein.

13) Method according to claim 12, characterised in that the support material is chosen from the group containing polymers, biopolymers, glass and composites containing silicone dioxides, metals and metal oxides, as well as any combination thereof on the microscopic, mesoscopic or macroscopic length scale.

14) Method according to any one of claims 12 and 13, characterised in that the support material is chosen from the group containing polymers, silicon dioxides, aluminum oxides, titanium oxides, magnesium oxides, borates, metals and other metal oxides.

15) Method according to any one of claims 13 and 14, characterised in that the polymers are chosen from the group containing polyolefins such as polystyrene, polyacrylates, polymethyacrylates, polybutylene, polyvinylalcohol and related derivatives, polyvinylchlorides, polyisoprene, polypropylene, polyphenols, polyamides, polyesters polysulfones, polyethersulfones, polyethersulfides, polyimines, polyethyleneglycols, polypropyleneglycols, polyimides, polycarbonates, polyurethanes,

16) Method according to claim 15, characterised in that the polymer surface is chemically treated to bear various functional groups chosen between carboxyl groups, hydroxyl groups, amino groups, amide groups, ester groups, imide groups, imine groups, mercapto groups, nitro groups, sulfonate groups, phosphate groups, phosphonate groups, cyano groups, sulfone groups, aldehyde groups, epoxide groups, urethane groups, ketone groups, phenolic groups, aromatic groups, alkyl, alkenyl, alkynyl, acyl and aryl groups, silanol groups, silicon oxide groups, siloxane groups, metal hydroxide groups, metal oxide groups, and elemental metals.

17) Method according to any one of claims 13 to 16, characterised in that the support material is carboxylated polystyrene.

18) Immobilised protein construct obtained by the method according to any one of claims 13 to 17, characterised in that it is covalently or non-covalently bonded to the support material.

19) Immobilised protein construct according to claim 18, characterised in that it is non-covalent and yet freely accessible and leach-free like proteins immobilised in the covalent sense.

20) Use of the immobilised protein constructs according to any one of claims 18 and 19 in applications selected from analysis, diagnosis (like in enzyme based diagnostic kits), incubation, storage, sensing, arraying and orienting, catalysis, stabilisation, binding, signal transduction, chemical transformation, implant passivation and surface biocompatibilization, surface activation, purification, detoxification and scavenging.

[Received by the International Bureau on 04 DEC 2003 (04.12.03) ; original claims 1 to 3, unchanged ; original claims 4 to 16, amended ; claims 17 to 20, cancelled]

1) Circular recombinant plasmid DNA construct encoding a) a protein tag, b) a visual marker protein, and containing c) a multiple cloning site suitable for insertion of an additional gene, characterised in that the gene sequence encoding the protein tag and the visual marker protein are specifically designed and engineered at the DNA level for respectively a) immobilisation purposes and b) visualisation and quantification purposes at the protein level.

2) Construct according to claim 1, characterised in that the protein tag is chosen from the group containing lysine (lys), histidine (his), tyrosine (tyr), phenylalanine (phe), arginine (arg), glutamic acid (glu), aspartic acid (asp), glutamate, aspartate, asparagine (asn), glycine (gly), glutamine (gln), alanine (ala), valine (val), tryptophan (trp).

3) Construct according to claim 1 or 2, characterised in that the protein tag is a histidine-tag such as a polyhistidine variant, in particular (6X) histidine.

4) Construct according to any one of the preceding claims, characterised in that the visual marker protein is chosen from the group containing fluorescent or phosphorescent proteins, wherein the fluorescent protein is chosen from the group containing Green Fluorescent Protein (GFP), Red Fluorescent Protein (RFP), Yellow Fluorescent Protein (YFP) and Blue Fluorescent Protein (BFP) as well as their variants and/or mutants.

5) Construct according to any one of the preceding claims, characterised in that the multiple cloning site contains restriction enzyme recognition sites chosen from the group containing SacI, Sal I, Hid III, Eag I, Not I.

6) Construct according to any one of the preceding claims, characterised in that it further contains a frame adapter of variable length between the visual marker and protein tag genes.

7) Construct according to any one of the preceding claims, characterised in that it expresses a fusion protein, wherein the tag is suitable to interact directly with appropriate surface pendant groups of a support matarial.

8) Construct according to claim 9, characterised in that the direct interaction with the support material is covalent or non-covalent.

9) Construct according to claim 10, characterised in that the direct interaction is non-covalent and yet freely accessible and leach-free like covalent one.

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10) Method for preparing and immobilising a protein on a support material, characterised in that it contains the steps of:
    a) Engineering at the DNA level, in series a protein tag suitable to interact directly with appropriate surface pendant groups of a support material, a fluorescent marker protein for visualisation and quantification purposes at the protein level and a multiple cloning site suitable for insertion of a target protein to be immobilised,
    b) Inserting the corresponding gene of the target protein to be immobilised into the multiple cloning site;
    c) Initiating protein expression.
    d) Optionally pre-treating the support material;
    e) Incubating the protein and support material together, wherein the protein is immobilised to the support via specific tag-surface interactions;
    f) Washing away the non-specific biomolecules;
    g) Optionally quantifying the fluorescence of the visual marker protein;
    h) Optionally desorbing the target protein.

11) Method according to claim 10, characterised in that the support material is chosen from the group containing polymers, biopolymers, glass and composites containing silicone dioxides, metals and metal oxides, as well as any combination thereof on the microscopic, mesoscopic or macroscopic length scale.

12) Method according to any one of claims 10 and 11, characterised in that the support material is chosen from the group containing polymers, silicon dioxides, aluminum oxides, titanium oxides, magnesium oxides, borates, metals and other metal oxides.

13) Method according to any one of claims 11 and 12, characterised in that the polymers are chosen from the group containing polyolefins such as polystyrene, polyacrylates, polymethyacrylates, polybutylene, polyvinylalcohol and related derivatives, polyvinylchlorides, polyisoprene, polypropylene, polyphenols, polyamides, polyesters polysulfones, polyethersulfones, polyethersulfides, polyimines, polyethyleneglycols, polypropyleneglycols, polyimides, polycarbonates, polyurethanes,

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14) Method according to claim 13, characterised in that the polymer surface is chemically treated to bear various functional groups chosen between carboxyl groups, hydroxyl groups, amino groups, amide groups, ester groups, imide groups, imine groups, mercapto groups, nitro groups, sulfonate groups, phosphate groups, phosphonate groups, cyano groups, sulfone groups, aldehyde groups, epoxide groups, urethane groups, ketone groups, phenolic groups, aromatic groups, alkyl, alkenyl, alkynyl, acyl and aryl groups, silanol groups, silicon oxide groups, siloxane groups, metal hydroxide groups, metal oxide groups, and elemental metals.

15) Method according to any one of claims 13 to 16, characterised in that the support material is carboxylated polystyrene.

16) Method according to claim 10, characterised in that quantifying in step g) of the fluorescence of the visual marker protein is used in applications selected from analysis, diagnosis (like in enzyme based diagnostic kits), incubation, storage, sensing, arraying and orienting, catalysis, stabilisation, binding, signal transduction, chemical transformation, implant passivation and surface biocompatibilization, surface activation, purification, detoxification and scavenging.

AMENDED SHEET (ARTICLE 19)